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An Alternative Method for the Comparative Study of Coronary Vessels: Repletion and Diaphanization Experience in Seven Animal Models

Repleción y Diafanización como una Alternativa para el Estudio Comparativo de los Vasos Coronarios: Experiencia en Siete Modelos Animales

Pedraza Rodriguez, Juan Daniel[1]; Muñetón, Cesar[2]; Melo, Fernando[3] & Rueda-Esteban, Roberto Javier[4]

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SUMMARY: Coronary arteries establish a complex blood vessel system, right and left coronary arteries commonly originate from the aortic sinuses and divide into multiple branches that supply the heart with several important variations between species. Diaphanization is a preservative technique which allows internal structures visualization, maintaining the three-dimensionality of the specimen. In this study, human (*Homo sapiens*), goat (*Capra aegagrus hircus*), bovine (*Bos Taurus*), equine (*Equus caballus*), porcine (*Sus scrofa domesticus*), canine (*Canis lupus familiaris*) and feline (*Felis silvestris catus*) coronary arteries were injected with self-curing methyl-methacrylate and posteriorly diaphanized. The coronary vasculature was adequately observed in all models while keeping the three-dimensional relation with surrounding cardiac structures, except for septal arteries which were not visualized. As incidental findings, anatomical variations in canine and human hearts were observed. Repletion-diaphanization is a useful blended method to visualize the morphology of superficial coronary arteries. It could be a valuable tool in anatomical teaching and research, but further research needs to be done to prove its effectiveness in different vessel systems.

KEY WORDS: Anatomy, Comparative; Anatomy, Regional; Coronary Vessels.

INTRODUCTION

Coronary arteries establish a system of vessels that supply hearts´ metabolic requirements. During diastole these arteries are filled with blood, which flows to each cardiac structure through several branches (Crick *et al*., 1998; Drake *et al*., 2010; Iaizzo, 2015).

Multiple methods have been used to describe the coronary paths and ramifications, such as direct dissection after formaldehyde fixation Crick *et al*., dissectible casting after polymeric repletion (Moore, 1930; Blair, 1961; Ozgel *et al*., 2004; Moura Junior *et al*., 2009; Oliveira *et al*., 2010; Gómez & Ballesteros, 2013); cardiac tissue digestion with corrosive agents (Blair; Bertho & Gagnon, 1964; Roldán & Blanquez Layunta, 1982; Weaver *et al*., 1986), clearing with wintergreen oil (Abramson *et al*, 1933; Moore *et al*., 2008) and radiological tools applied to obtain post mortem images of the heart (Rodrigues *et al*., 2005). However, polymeric repletion and subsequent diaphanization of adult hearts have not been presented as a tool to compare coronary paths and/or ramification between species. This technique can be useful for anatomy teaching Rueda-Esteban *et al*. (2017).

The aim of this study is to determine if repletion-diaphanization is a useful tool to improve superficial coronary anatomy visualization.

Brief anatomical description

Human (*Homo sapiens*), goat (*Capra aegagrus hircus*), bovine (*Bos taurus*), equine (*Equus caballus*), porcine (*Sus scrofa domesticus*), canine (*Canis lupus familiaris*) and feline (*Felis silvestris catus*) hearts were

[1] Medicine student, Universidad de los Andes School of Medicine, Anatomy for Education Research Group, Colombia.
[2] Assistant Professor, Universidad de la Salle School of Veterinary Medicine, Colombia.
[3] Cathedra Professor, Fundación Universitaria Agraria de Colombia School of Veterinary Medicine. Universidad de la Salle School of Veterinary Medicine, Colombia.
[4] Assistant Professor, Anatomy Coordinator, Universidad de los Andes School of Medicine, Anatomy for Education Research Group, Colombia.

selected due to their anatomical importance in veterinary and human medicine teaching and research.

For human, the left coronary artery (LCA) divides into the anterior descending artery and the circumflex branch (CxB). Their branches supply mainly the left atrium and ventricle. In contrast, the posterior descending artery (PDA) and right marginal artery (RMA) emerge from the right coronary artery (RCA), which (along with other branches) allow blood flow to the right heart and a portion of the left ventricle (Rodriguez *et al*., 1961; Moore *et al*.; Drake *et al*.).

Porcine and feline LCA divides into the paraconal interventricular branch (PIB) and CxB. With other branches, these vessels supply the left atrium and ventricle. On the other hand, the subsinusoidal branch (SB) and RMA rise from the RCA. The branches of these vessels supply the right heart and a part of the left ventricle. However, in cited literature RMA is not reported for felines´ cardiac anatomy (Reighard & Jennings, 1901; Weaver *et al*.; Abramson *et al*.; Crick *et al*.; Gómez & Ballesteros; Rodrigues *et al*.; Rodriguez *et al*.; Sahni *et al*., 2008).

For bovines and equines, the RCA is called right circumflex artery (RCx) after it emerges. In both species, the LCA divides into the PIB and CxB, but SBs´ origin is different, in bovines this arterial branch is a ramification of the LCA while in equines it is from the RCA (Roldán & Blanquez Layunta; Gloobe, 1989; Budras & Budras, 2003; Ozgel *et al*.; König & Liebich, 2008).

In canines, the right heart is supplied by the RCA, which in its path is called RCx and originates the RMA. The left heart with part of the right ventricle is supplied by the CxA, PIB and SB; branches of the LCA (Moore; Besso Pianetto, 1939; Blair; Oliveira *et al*.; Evans & De Lahunta, 2013). Goats´ coronary anatomy share distribution similarities with canines; however, RCx is not reported in them (Yang *et al*., 1989; Moura Junior *et al*.).

Finally, the septal arteries cross the interventricular wall supplying it. In canine, bovine and goat this vessel has been reported as a unique artery; while in human, porcine, equine, and feline it is defined as an anastomosis of branches from the LCA and RCA (Abramson *et al*.; Bertho & Gagnon; Yang *et al*.).

MATERIAL AND METHOD

Seven adult hearts, one of each species, were used for this study. Age, size, weight and sex were not considered relevant. The human heart was acquired at the Universidad de los Andes School of Medicine Anatomy Laboratory, under the Research Ethics Board Act No. 448 of 2015 and the Expedited Endorsement for cadaveric dissection and description Act No. 518 of 2015. Porcine, bovine, and goat hearts were obtained as products of food industry. Canine, feline, and equine specimens were obtained after euthanasia due to medical conditions with the appropriate indication of this procedure, non-related to this study. Animal specimens were used under the approval of our Institutional Animal Care and Use Committee (IACUC) (Reference: CICUAL_17-021).

Repletion was carried out by injecting methyl-methacrylate through the coronary arteries, beginning at the ascending aorta. The injection of the atrial and ventricular cavities was not required. Fixation and dehydration were done by submersion in ethylic alcohol at increasing concentrations until 96.9 % was reached. Diaphanization was then carried out in all specimens following our Anatomy Laboratory protocol Rueda-Esteban *et al*. Potassium hydroxide (KOH) at an initial concentration of 4-5 % was used to macerate the tissue. Then, the hearts were cleared with anhydrous glycerin and KOH, varying the glycerin-KOH proportion until the specimen was totally immersed in glycerin. Finally, pictures of the hearts were taken, and the background was eliminated with Macromedia Fireworks 8 and Adobe Photoshop 10, without editing the hearts.

RESULTS

All hearts were successfully injected, six of them were partially diaphanized (bovine, equine, porcine, canine, human and goat) and one fully diaphanized (feline). Despite this, in some species the epicardial fat distribution didn´t allow proper visualization of some coronary arteries (Fig. 1). The observed anatomy was concordant with the literature, however as incidental findings, two anatomical variations were found: in the human heart, the PDA emerged from the LCA; in canine heart, the SB was a branch of the RCA.

DISCUSSION

Big hearts need a longer maceration time to complete the diaphanization process. However, coronary paths and its relationship with other cardiac structures can be seen through partial diaphanization. For Bovine and equine hearts, the CxB and RCx cannot be easily visualized because they are surrounded by epicardial fat (Fig. 1), which can be removed through dissection before the diaphanization process,

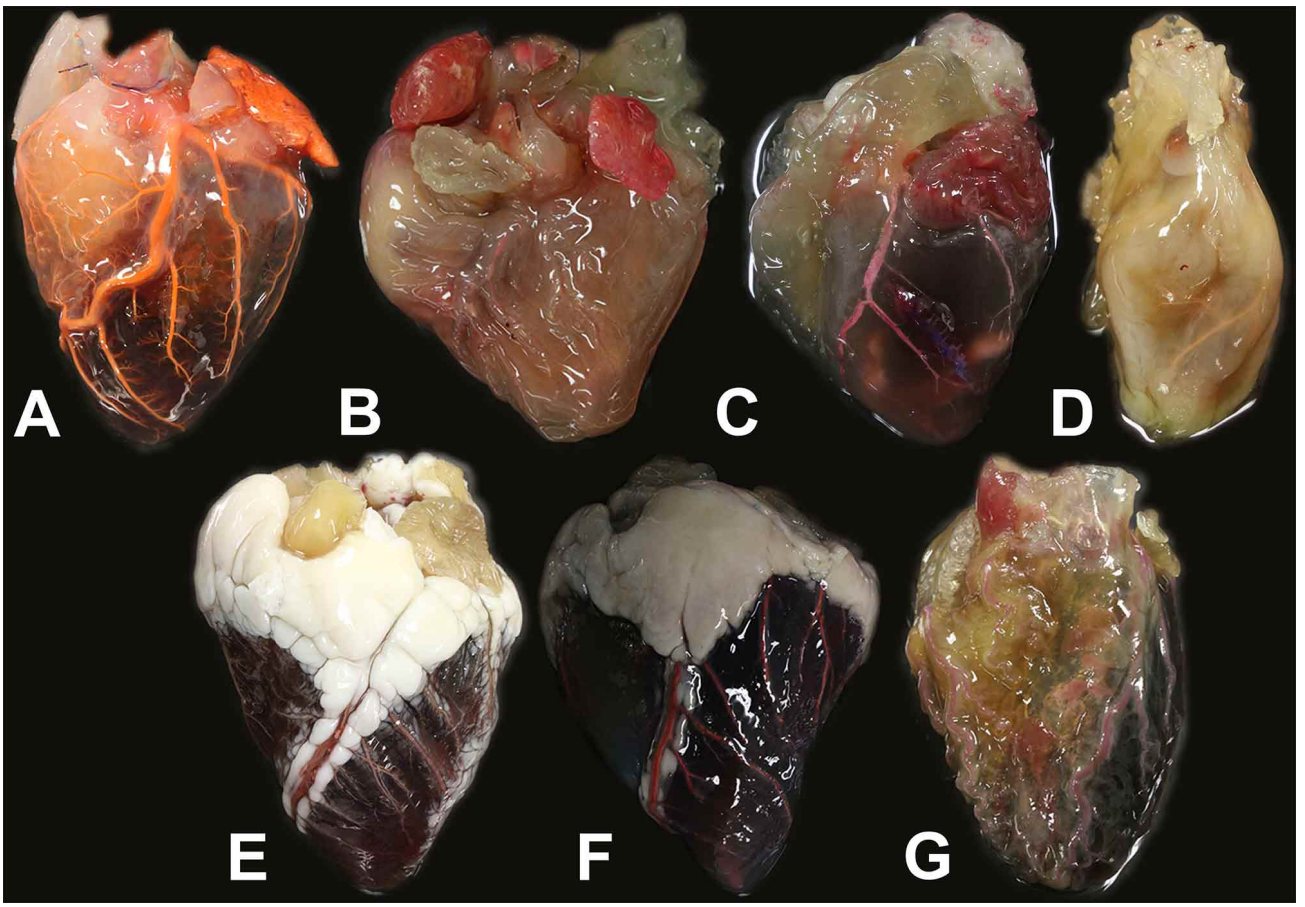

Fig. 1. Ventral view of each heart. A. Porcine, B. Goat, C. Canine, D. Feline, E. Bovine, F. Equine, G. Human.

not pursued for this study. Septal arteries are not correctly visualized through this technique, since full maceration is necessary, increasing the risk of tissue damage.

By blending the approaches of repletion to highlight coronary vessels and transparency obtained through diaphanization (even if partial), repletion-diaphanization proves to be a useful tool to observe main superficial coronary arteries without damaging related cardiac structures and three-dimensional arrangement of the heart. The use of this type of specimens could be a valuable tool for teaching and research purposes. Further research should be done to determine its functionality and shelf life.

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RESUMEN: Las arterias coronarias son un complejo de vasos sanguíneos que usualmente se originan en los senos aórticos y que al dividirse en múltiples ramas suplen los requerimientos metabólicos del tejido cardiaco; cabe aclarar que la anatomía de estas estructuras posee variaciones importantes entre especies. La diafanización es una técnica de preservación que permite observar estructuras internas de un espécimen sin dañar su tridimensionalidad. En este estudio las arterias coronarias del corazón humano (*Homo sapiens*), caprino (*Capra aegagrus hircus*), bovino (*Bos Taurus*), equino (*Equus caballus*), porcino (*Sus scrofa domesticus*), canino (*Canis lupus familiaris*) y felino (*Felis silvestris catus*), fueron repletadas con metil-metacrilato y posteriormente diafanizados. Se observa la irrigación coronaria de cada uno de los corazones y su relación con las demás estructuras cardiacas, exceptuando las arterias septales. Como hallazgos incidentales se observaron variaciones anatómicas en los corazones canino y humano. Finalmente, esta técnica resultó de utilidad para evaluar la anatomía coronaria, lo que puede ser valioso para educación e investigación. Posteriores investigaciones deben ser realizadas para probar su utilidad en otros sistemas vasculares.

PALABRAS CLAVE: Anatomía; Comparativa; Anatomía; Regional; Vasos coronarios.

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Corresponding author:
Roberto J. Rueda E. M.D. M.Ed. M.Sc.
Assistant Professor
Anatomy Coordinator
Universidad de los Andes School of Medicine
Zip-code: 111711
Bogotá D.C.
COLOMBIA

E-mail: rj.rueda32@uniandes.edu.co

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